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Multisite evaluation of the BD™ Stem Cell Enumeration (SCE) Kit for CD34+ cell enumeration on the BD FACSCanto II™ and BD FACSCalibur™ flow cytometers

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Abstract

Background—Evaluation of the BD™ Stem Cell Enumeration (SCE) Kit was conducted at four clinical sites with flow cytometry $CD34^+$ enumeration, to assess agreement between two investigational methods, the BD FACSCanto™ II and BD FACSCalibur™ systems, and the predicate method (Beckman Coulter Stem-Kit™ reagents).

Methods—Leftover and delinked specimens (n = 1,032) from clinical flow cytometry testing were analyzed on the BD FACSCanto II (n = 918) and BD FACSCalibur (n = 905) in normal and mobilized blood, frozen and thawed bone marrow, and leucopheresis and cord blood anticoagulated with CPD, ACD-A, heparin, and EDTA alone or in combination. Fresh leucopheresis analysis addressed site equivalency for sample preparation, testing, and analysis.

Results—The mean relative bias showed agreement within predefined parameters for the BD FACSCanto II (−2.81 to 4.31 ±7.1) and BD FACSCalibur (−2.69 to 5.2 ±7.9). Results are reported as absolute and relative differences compared to the predicate for viable $CD34^+$, percentage of $CD34^+$ in $CD45^+$, and viable $CD45^+$ populations (or gates). Bias analyses of the distribution of the predicate low, mid, and high bin values were done using BD FACSCanto II optimal gating and BD FACSCalibur manual gating for viable $CD34^+$, percentage of $CD34^+$ in $CD45^+$, and viable

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In memory of Rudy Varro, our R&D colleague, for his contributions and dedication to this BD SCE program.

Disclosures of Interest

The accuracy study was sponsored by BD Biosciences. Data were used by the sponsor to support regulatory submission in Europe and the US, and worldwide market distribution.

Robert Preti, Joanne Kurtzberg, Paul K. Wallace, Thorsten Tonn, Wai-shun Chan, Ronna Dornsife, Rosemary Furlange, and Halvard Bonig have no financial interest to disclose.

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$CD45^+$. Bias results from both investigational methods show agreement. Deming regression analyses showed a linear relationship with R^2 >0.92 for both investigational methods.

Discussion—In conclusion, the results from both investigational methods demonstrated agreement and equivalence with the predicate method for enumeration of absolute viable $CD34^+$, percentage of viable $CD34^+$ in $CD45^+$, and absolute viable $CD45^+$ populations.

Keywords

CD34; engraftment; flow cytometry; progenitor cells; stem cells; transplantation

Introduction

Stem and progenitor cells are obtained from diverse sources, including bone marrow (BM), peripheral blood (PB), and cord blood (CB), for both autologous and allogeneic use in a growing number of therapeutic indications. Traditionally, the majority of autologous and allogeneic hematopoietic stem/progenitor cell (HSPC) transplants were performed utilizing BM as a source of stem cells. However, by the end of the 1980s major improvements in the harvesting of peripheral blood stem cells (PBSC) were attained (1). Data demonstrating that the time required for hematopoietic reconstitution is significantly shorter with PBSC than BM has led to the widespread use of PBSC for autologous and, increasingly, allogeneic transplantation (2). In parallel, CB has been a source of HSPC (3), and numbers of successful transplants have grown significantly worldwide since the first transplant in 1988. Since that time, the exploding fields of cellular therapy and regenerative medicine have relied upon stem and progenitor cells from adult and embryonic sources alike to advance the science of and unlock the unlimited potential for these cells to cure a wide variety of diseases for which heretofore only palliative treatments of symptoms have been available.

Since its discovery almost 30 years ago (4,5) the CD34 antigen, expressed on BM-derived progenitor/stem cells, which also appears in PB and CB as well, has been increasingly relied upon to identify a subpopulation of cells with regenerative capacity for hematopoiesis, angiogenesis, and lymphopoiesis, to name a few. Perhaps more than any other single cell type, the $CD34^+$ cell has been the subject of intense investigation, both clinical and preclinical, and has been quantified, qualified, expanded, selected, and genetically modified due to its wide-ranging potential to serve as a platform for cellular therapy and regenerative medicine applications.

Flow cytometry is a universally accepted and widely employed methodology for evaluation of stem and progenitor cell products for content of a variety of cellular types and subsets. Given the central role of the CD34 antigen, the focus on the cells that carry this antigen has been intense in the development of this exciting new class of cellular-based therapeutic.

As with all analytical methods, the accuracy, precision, reliability, and other measures of the quality of the method are challenged at limits of the analysis spectrum. At the extremes at each end of this spectrum (low and high concentrations of the analyte being tested), all quality control methods lose performance, and at some point data are reported as being “beyond the limit of detectability.” Therefore, as a consequence of the combination of the

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$CD34^+$ cell's central importance and rare event nature, analysis of this cell at the low end of detectability has been the focus of a large number of scientific, regulatory, and technical investigations. Getting the quantity of these cells right allows for the proper dosing to enable them to affect their mechanism of action.

Sutherland et al (6) developed a multiparametric method combining forward and side light scatter with the intensity of $CD34^+$ and $CD45^+$ fluorescent antibody staining to give sensitive and accurate $CD34^+$ cell enumeration of these rare events. Analysis was conducted by applying a Boolean gating strategy, coupled with cluster analysis amenable for use on hematopoietic stem cells from various sources. This sequential gating method, which formalized a standardized format through the use of which both interlaboratory and intralaboratory comparability could be assured and importantly one can eliminate the compounding variability consequent to the use of dual-platform analyses, formed the basis of a clinical guideline for $CD34^+$ cell enumeration from the International Society for Cellular Therapy (ISCT) (formerly International Society of Hematotherapy and Graft Engineering or ISHAGE) (7) and is a featured method in *Current Protocols in Cytometry* (8). The method has since been widely used and validated (9). Since flow cytometric enumeration of $CD34^+$ cells plays a critical role in identifying the optimum product for transplantation, this technology will continue to have a major role in defining and evaluating the most suitable product in an increasingly diverse set of transplantation therapies (10).

BD Biosciences has recently developed a single platform (11, 13, 15) stem cell enumeration assay for the BD FACSCanto II, that incorporates BD Trucount™ counting beads and viability stain, the BD Stem Cell Enumeration Kit. This kit was introduced in the European market, and results with this kit were published in *Cytotherapy* (14).

The objective of the study reported in *Cytotherapy* was to evaluate the agreement between two investigational methods, BD FACSCanto II and BD FACSCalibur, and a predicate method, the Beckman Coulter, Inc. using the (BCI) Stem-Kit. The predicate method is defined as the previously IVD cleared or generally accepted method and is sometimes referred to as the reference method. Therefore, a demonstration of the agreement with the predicate method indicates accuracy of the investigational method and provides assurance to the user that a novel method for quality control (QC) testing has the necessary reliability to produce meaningful and comparable data. Importantly, agreement serves as a required demonstration for regulatory clearance from the US Food and Drug Administration (FDA) to market a device for a stated purpose.

The evaluation of the investigational BD SCE Kit on the BD FACSCanto II and BD FACSCalibur flow cytometers was conducted in two stages.

The European Performance Evaluation (EPE) was started in 2009 and expanded into a second stage, following a protocol amendment, to incorporate the US sites in 2010. The intra-site results from the EPE, which included one site in the EU, were published in 2011 and showed that results from both assays were highly congruent, with an overall $R^2 \geq 0.99$ (14).

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These results encouraged the continuation of the study and expansion into the US. In this current study, three additional sites were engaged to confirm the intra-site consistency demonstrated in the EPE, and to perform inter-site comparability testing to evaluate the robustness of the method to sustain multisite comparison. This manuscript describes the results from the BD SCE Kit accuracy evaluation at multiple sites using flow cytometry to enumerate the $CD34^+$ cells within in $CD45^+$ cell populations.

Materials and Methods

Study Sites and Study Design

The study was conducted at four sites, including representation from both the EU and the US, external to BD Biosciences, the sponsor of the study. The following institutions (sites) participated in the study: the German Red Cross (DRK) in Frankfurt, Germany; Duke University (DUK) in Durham, NC; Progenitor Cell Therapy (PCT) in Allendale, NY; and Roswell Park Cancer Institute (RPC) in Buffalo, NY.

All participating investigators and site staff were experienced in methodologies for stem cell collection and processing, which are integral activities in their regular laboratory work. DRK is located at the Johann Wolfgang Goethe University Campus in Frankfurt, in collaboration with the Institute for Transfusion Medicine and Immunohematology, and has a strong program in experimental and clinical stem cell research. DUK, specifically the Stem Cell Laboratory from the Pediatric Blood and Marrow Transplant Program, supports clinical services and research for processing and banking of CB units. PCT, a division of Neostem, an independent company focused on cell therapy services, has long experience in cell therapy development, manufacturing, and analysis over a wide range of different cell types. RPC, as a member of the National Comprehensive Cancer Network, possesses strong clinical and research programs in blood and marrow stem transplantation. Participating site staff routinely performed flow cytometric enumeration of hematopoietic stem cells and evaluated specimens for eligibility in this study.

Informed Consent and Approvals

All sites received Institutional Review Board (IRB) or Ethics Committee (EC) approval to proceed prior to initiation of investigational activity.

Inclusion/Exclusion Criteria

Specimens that provided valid results were delinked from the subject from whom the specimen was drawn and were required to have been collected and handled prior to enrollment in accordance with each site's policies and procedures. All specimen volumes (post-dilution volume, when applicable) were required to be ≥ 800 μL. For fresh specimens, storage at 2° to 8°C from the time of receipt in the testing laboratory to the time of enrollment and staining (maximum 24 hours from draw) was required. For frozen specimens, enrollment and staining were to be performed as soon as practicable and within one hour of thaw. When required, WBC counts were adjusted by dilution just prior to staining in cold phosphate buffered saline (PBS) with 0.5% bovine serum albumin (BSA) to $<30 \times 103$ cells/μL.

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Normal peripheral blood (NPB) and mobilized peripheral blood (MPB) samples were required to be remnant material from routine laboratory testing and anticoagulated with ethylenediaminetetraacetate (EDTA). Fresh or frozen CB and leucopheresis (LPH) specimens also were required to be left over from flow cytometry $CD34^+$ enumeration and were required to be anticoagulated with EDTA, heparin, citrate phosphate dextrose (CPD), and/or anticoagulant citrate dextrose – solution A (ACD-A). Fresh or frozen BM specimens, left over from lymphoma staging or from flow cytometry $CD34^+$ enumeration, were required to be anticoagulated with EDTA, heparin, CPD, and/or ACD-A, and have no indication of presence of malignant $CD34^+$ cells (ie, presumptive leukemia or myelodysplastic syndrome).

Enrolled Sample Acquisition

Only leftover specimens, defined as remnants of human specimens not used for a test, which would otherwise have been discarded, were evaluated for eligibility. Leftover and delinked specimens from routine flow cytometric $CD34^+$ enumeration that met inclusion criteria and none of the exclusion criteria were enrolled in the study.

Samples prepared with the investigational BD SCE Kit or predicate kit were acquired on instrumentation as described in Table 1.

Enrolled Sample Preparation

Samples were prepared according to the manufacturer's instructions included with each kit, ie, the BD SCE Kit or the BCI Stem-Kit.

On the day of the study, instrument setup was completed prior to running process controls. The BD FACSCanto II was set up with BD FACS™ 7-color setup beads, followed by spillover optimization, and the BD FACSCalibur was set up with BD Calibrite™ beads. Process controls achieved acceptable results prior to specimen enrollment. The BD Stem Cell Control kit and BCI Stem-Trol™ control cells were prepared according to the manufacturer's instructions.

Specimens were prepared as duplicate samples (4 tubes) for both investigational methods. For the predicate, specimens were prepared as duplicate samples, along with one isoclonic control (3 tubes). Sample preparation of the investigational method consisted of adding 20 μL of BD SCE reagent (CD45-FITC/CD34-PE) and 20 μL of 7-aminoactinomycin D (7-AAD) to the 100 μL of sample, vortexing, and incubating for 20 minutes in the dark. NH_4Cl solution (1X, 2 mL) was added, and the tubes were vortexed and incubated for another 10 minutes in the dark.

At the end of the lysing period, samples were transferred to melting ice, protected from light, and acquired within one hour. Samples were re-vortexed immediately before acquiring. For the predicate method, 20 μL of the BCI Stem-kit reagent [CD45-FITC/CD34-PE] and 20 μL of 7-AAD were mixed with 100 μL of sample. A third tube with the 20 μL of isoclonic control and 100 μL of sample was prepared, vortexed, and incubated for 20 minutes in the dark, followed by addition of NH_4Cl lysing solution (1X, 2 mL). The tube was vortexed and incubated for 10 minutes in the dark. At this point, 100 μL of BCI Stem-

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Count™ fluorospheres were dispensed, stored on melting ice in the dark, and acquired within 1 hour. Samples were re-vortexed immediately before acquiring.

Samples were acquired with the BD FACSCalibur using the BD SCE template and with the BD FACSCanto™ clinical software application module. Both analysis methods were developed based on the ISHAGE modified gating strategy illustrated in Figure 1.

To eliminate operator-dependent and operational biases, a schedule for sample acquisition was devised such that the order of acquisition between the different instruments and each operator's use of each instrument were routinely interchanged.

Specimen Population and Distribution—"Bins"

The target number of specimens was a minimum of 850 enrolled specimens with valid results for each investigational method. Results were valid when the data were accurate, complete, and in compliance with all applicable protocol requirements. It was anticipated that 1,020 specimens (an additional 20%) might have been required be enrolled to achieve the required 850 specimens with valid results distributed by specimen types.

To be evaluated as part of the analysis sample pool and meet the primary endpoint for each investigational method, three prospectively determined criteria had to be satisfied. The specimens were assigned to bins according to the measured viable $CD34^+$ cells (Abs CD34) of the predicate method, and the values were required to fit into one of three ranges (bins): low, mid, and high. "Low" was defined as a range of ≤10 cells/μL; "mid" as a range of >10 but ≤100 cells/μL, and "high" as a range of >100 but ≤1,000 cells/μL, as assessed by the predicate. Similarly, the specimens were assigned to low and high bins of percentage of $CD34^+$ in CD45 (%CD34) based on the predicate measured value. The low bin was required to be ≤0.5%, and high bin was required to be >0.5%. Last, there were no binning assignments based upon measured viable absolute $CD45^+$ (Abs CD45).

Sites enrolled eight types of leftover specimens: NPB, MPB, fresh and thawed LPH products, fresh and thawed BM, and fresh and thawed CB specimens. The samples were anticoagulated with CPD, ACD-A, heparin, and/or EDTA alone or in combination.

Determination of Site Equivalency

To prevent inter-site variability due to inadequate technical proficiency, training of staff at each site was performed by BD Biosciences staff, and was followed by technical qualification and equivalency analysis between the sites. Fresh LPH was used to show site equivalence on procedures for sample preparation, testing, and analysis. Data were analyzed and reported as absolute and relative differences compared to the predicate for measured viable $CD34^+$. Determination of equivalency of each site was required to allow for poolabilty of the data to analyze the study dataset in its entirety, independent of the site at which each sample was analyzed.

Specimens Excluded from Analysis

Prior to final analysis, data were reviewed, and some enrolled specimens were deemed not to be evaluable because they did not meet the eligibility requirements to allow entry into the

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dataset. The most common reasons for exclusion from analysis were non-compliance with inclusion criteria or protocol procedures.

Evaluation of Investigational vs Predicate Method

Bias analyses were performed based on distribution of predicate values in low, mid, and high bins using optimal gating for the BD FACSCanto II and manual gating for the BD FACSCalibur instrument.

On each day of study testing, fresh NPB and MPB, fresh and thawed LPH specimens, fresh and thawed BM, and fresh and thawed CB were evaluated according to the inclusion/exclusion criteria for this study listed previously (see Inclusion/Exclusion Criteria). Fresh samples were enrolled and stained within 24 hours post-draw. Frozen specimens were enrolled and stained as soon as practicable and within 1 hour post-thaw. If a specimen met all criteria, the enrollment process was documented on the appropriate case report form (CRF) and the specimen was delinked from the diagnostic specimen and or subject identification by assignment of a study-specific specimen number.

Evaluation of Inter-site Variabilty

To further evaluate the robustness of the investigational as well as predicate methods, we compared the results for each site by specimen type and method. It is important to note the limitations of this analysis, since the specimens evaluated at each site were analyzed only at that particular site, and in no case was an analysis of a single sample performed at more than one site. Therefore, direct comparability analysis between the sites was confounded by the difference in specimens at the site, since the specimens might be procured and processed following procedures affected by local practices. Therefore, of particular interest are the data generated from the analysis of specimen types that were less readily influenced by these local practices, such as NPB and fresh CB.

Statistical Analyses

The concept of accuracy includes both bias (systematic error) and precision (random error), where bias is defined as a persistent positive or negative deviation of the measured value from the true, or in this case predicate, value. To evaluate agreement of the test method with the predicate, we devised an experimental design to evaluate the mean bias or difference (terms that are used interchangeably) in each of the cell-concentration bins, after control of random error through training of analytical personnel, test site qualification, demonstration of site equivalency, and required operational flow to balance various testing patterns. Further, we balanced the number of sample types collected at each testing site involved in the study.

Prior to analysis, site equivalency (ie, poolability) was evaluated using the mean bias of the LPH specimen from each site for each study method before pooling all data across sites. For each investigational method, the mean bias between all sites must have fallen within the poolability criteria for site equivalency.

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All bias and regression analyses were performed using measured versus reported values of the investigational methods versus measured values of the predicate method. The measured and reported values differ if specimens were diluted during sample preparation. The reported value refers to the measured value multiplied by the dilution factor. Concordance analyses were performed on reported values.

Each specimen was analyzed separately for Abs CD34, %CD34, and Abs CD45 results. Data from the first replicate of the investigational methods from each specimen were used for the bias calculation between the investigational and predicate methods. Data from the second replicate of the investigational methods from each specimen were used for the outlier determination. For each specimen, the CD34 bins were determined based on the Abs CD34 and %CD34 results from the predicate system.

Study data were analyzed and reported as absolute difference and relative difference compared to the predicate for Abs CD34, %CD34, and Abs CD45. Site personnel optimized the automated algorithm data for the BD SCE Kit on the BD FACSCanto II by manual gating, if deemed necessary. These optimized results were used for endpoint analysis of this investigational method.

For each specimen, the bias or absolute or relative differences between the investigational and predicate systems (for Abs CD34, %CD34, and Abs CD45 results) were calculated and pooled across all specimens by bins, to produce a mean bias along with a 95% confidence interval (CI) for that range of CD34 values according to the EP9-A2-IR CLSI guideline (16, 18). The absolute difference and the relative difference were calculated using the following formulas:

$$Absolute\ Difference = Investigational - Predicate$$

$$Relative\ Difference = \frac{Investigational - Predicate}{Predicate} \times 100$$

Results

Specimen Population and Distribution

Study testing was performed on a total of 1,032 remnant enrolled specimens that had been delinked of their patient identification through the application of new labeling to replace previous identifiers. Of the total of 1,032 leftover and delinked specimens enrolled in the four clinical sites, the distribution of required and analyzed specimens by investigational method is shown in Table 2. The distribution of the required and analyzed specimens by investigational method, anticoagulant, and specimen type used in the study was well balanced between the two instruments. To be analyzed, matching evaluable results must have been available for the predicate method.

The anticoagulants used during the clinical study included CPD, ACD-A, heparin, and EDTA, and combinations such as heparin plus ACD-A and EDTA plus ACD-A (see Table 3).

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Fresh specimens within 24 hours of collection and frozen specimens within 1 hour of thawing were enrolled in the study. NPB and MPB were only fresh specimens; BM, CB, and LPH were fresh and frozen. The mean $CD34^+$ viability for NPB and MPB by method is presented in Table 4. Table 5 summarizes $CD34^+$ viability for BM, CB, and LPH by method.

Specimens Excluded from Analysis

During data cleaning, specimens were eliminated from analysis because they did not meet the data evaluability criteria or because during testing the protocol procedures were not followed. A total of 148 specimens did not provide valid results (DRK = 64; DUK = 21; PCT = 17; and RPC = 46). The reasons for exclusion were failure to meet inclusion criteria, inappropriate instrument setup or sample preparation, poor review or lack of review of the data of quality control criteria after acquisition, and instrument errors. The majority of samples that were excluded did not meet quality control criteria for viable $CD45^+$ events and/or viable $CD34^+$ events or acquisition time. Of the 148 specimens excluded, 81 specimens were completely excluded from the analysis because the specimen was disqualified from the predicate method. All the samples were automatically excluded from the BD FACSCanto II and BD FACSCalibur methods as well, since side-to-side comparison with the predicate was not feasible. Forty-six specimens were excluded from data analysis for the BD FACSCanto II alone and 21 specimens for the BD FACSCalibur alone.

Determination of Site Equivalency

Fresh LPH was used to show site equivalency on procedures for sample preparation, testing, and analysis. A minimum of 55 fresh LPH specimens were enrolled by site (see Table 6). The relative difference of the mean bias was within −2.81 to 4.31 for the BD FACSCanto II and −2.69 to 5.2 for the BD FACSCalibur, showing <10% difference between the sites, thereby meeting the predefined acceptance criteria. These results demonstrated that it was acceptable to pool the data for analysis from all four sites to lend power to the analysis of Abs CD34, %CD34, and Abs CD45 agreement for each specimen type between the investigational and predicate methods.

Agreement Analysis of the Investigational and Predicate Methods

The bias analyses of Abs CD34 were grouped in low, mid, and high bins to obtain the absolute and relative differences from the predicate in both investigational methods. Results are summarized in Table 7.

Low bin (<10 cells/μL)—The low bin included 167 and 156 specimens tested on the BD FACSCanto II and BD FACSCalibur. The means of Abs CD34 were 3.8 and 3.9 for the BD FACSCanto II and predicate, and 3.5 and 3.8 on the BD FACSCalibur and predicate, respectively. The absolute differences were −0.1 ±3.1 (−0.5, 0.3) for the BD FACSCanto II and −0.2 ±1.4 (−0.4, −0.05) for the BD FACSCalibur, respectively. The relative difference was not applicable for this bin.

Mid bin (>10 to <100 cells/μL)—In the mid bin, 496 and 492 specimens were analyzed on the BD FACSCanto II and the BD FACSCalibur, respectively. The mean values of Abs CD34 were 43.3 and 44 for the BD FACSCanto II and predicate, and 45 and 44.4 for the BD

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FACSCalibur and predicate, respectively. The absolute differences were −0.7 ±8.9 (−1.36, −0.035) for the BD FACSCanto II and −0.6 ±9.6 (−1.3, 0.2) for the BD FACSCalibur, respectively. The relative differences were −1.6 ±19.4 (−3.1, −0.2) and −0.9 ±18.7 (−2.3, 0.5) for the BD FACSCanto II and BD FACSCalibur, respectively.

High bin (>100 to <1000 cells/μL)—In the high bin, 255 specimens were tested on the BD FACSCanto II and 257 on the BD FACSCalibur. The means of Abs CD34 were 217.1 and 218.5 for the BD FACSCanto II and predicate and 217 and 213.5 on the BD FACSCalibur and predicate, respectively. The absolute differences were −1.4 ±28.7 (−4.3, 1.6) for the BD FACSCanto II and −3.6 ±31.5 (−6.8, −0.3) for the BD FACSCalibur, respectively. The relative differences were −1.0 ±13.5 (−2.4, 0.39) and −1.7 ±13.9 (−3.1, −0.2) for the BD FACSCanto II and BD FACSCalibur, respectively.

The values of %CD34 were grouped in low and high bins to determine the absolute and relative differences from the predicate on both investigational methods. In the low bin, there were 512 and 487 specimens, and the high bin included 406 and 418 specimens for the BD FACSCanto II and BD FACSCalibur, respectively.

%CD34 low bin—The absolute difference of %CD34 was −0.004 ±0.1 (−0.01, 0.0002) for the BD FACSCanto II and −0.002 ±0 (−0.01, 0.0002) for the BD FACSCalibur. The %CD34 relative difference was −2.2 ±29.2 (−4.4, −0.1) and −0.8 ±28.8 (−3.0, 1.3) for the BD FACSCanto II and BD FACSCalibur, respectively.

(Note: On the predicate system, 11 samples, DRK241, DRK242, DRK322, DRK323, DRK 325, DRK 331, DRK400, DRK289, DRK290, RPC192, and RPC022, had a value of zero (0) for viable CD34 and %CD34. Therefore, a total of 501 specimens were used to calculate the relative difference of the %CD34 low bin).

%CD34 high bin—The absolute difference values for %CD34 were 0.78 ±0.5 (0.04, 0.12) for the BD FACSCanto II and 0.016 ±0.9 (0.09, 0.24) for the BD FACSCalibur. The relative differences for %CD34 were 2.1 ±27.2 (−0.11, 4.3) and −3.2 ±28.3 (0.9, 5.5) for the BD FACSCanto II and BD FACSCalibur, respectively.

All specimens were analyzed for the relative difference for Abs CD45. The values were −1.7 ±21.2 (−2.8, −0.5) and −2.0 ±25.0 (−3.4, −0.6) for the BD FACSCanto II and BD FACSCalibur, respectively.

Most of the samples fell within the mid-bin range, followed by the high bin, and the low bin with the least number of samples in both investigational methods. The absolute differences by bin and method were close to each other. In similar fashion, the relative difference of Abs CD34 for the low and high bins by method was tight. Note that there were 11 specimens with “0” viable CD34 counts that were excluded for estimation of the relative difference of %CD34 for the BD FACSCanto II. Results from both investigational methods met the acceptance criteria.

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Regression Analysis

Regression analysis was performed by applying Deming regression (13) to Abs CD34, %CD34, and CD45 absolute difference from the site operator's optimized gating. In the regression plots, the values on the horizontal (x) axis represent the predicate method and the values on the vertical (y) axis represent the investigational method. The solid line in the plot is the identity line and the blue dotted line is the regression line. Figure 2 illustrates the Deming regression plots of the investigational and predicate methods for viable $CD34^+$ values from optimized gating. Results for both investigational methods show a linear correlation for all variables. For the total of 918 specimens analyzed on the BD FACSCanto II, the Abs CD34 had an R2 ≥0.94, a slope of ≥0.96, and an intercept ≥ −0.15; for %CD34, R2 was 0.92, the slope was ≥1.05, and the intercept was ≥ −0.05; for Abs CD45, R2 = 0.94, the slope was ≥0.98, and the intercept was ≥ −58.3. A total of 905 specimens were analyzed on the BD FACSCalibur, with an Abs CD34 with an R2 ≥0.95, a slope ≥0.97, and an intercept ≥ −0.11; for %CD34, R2 = 0.92, the slope was ≥1.06, and the intercept ≥ −0.04; for Abs CD45, R2 = 0.94, the slope was ≥0.97, and the intercept was ≥ −68.3.

Regression Analysis by Specimen Type

The regression analysis was completed by integrating values from BM, CB, and LPH fresh and frozen for both investigational methods, the BD FACSCanto II and BD FACSCalibur (BM = 148/151; CB = 241/245; LPH = 341/342). Peripheral blood (PB) was formed after pooling MPB and NPB in one single group (PB = 188/167). Results show a linear correlation for the Abs CD34, with R^2 between 0.88 and 1.00 for the BD FACSCanto II, and between 0.94 and 0.97 for the BD FACSCalibur (Figure 3).

Concordance Analysis

Concordance analysis was performed using the reported values to assess the percentage of agreement between the investigational and predicate methods at the predefined clinical decision cut-off value of 10 $CD34^+$ cells/μL. The numbers of samples for the BD FACSCanto II and BD FACSCalibur included in the analysis were 915 and 905, respectively. The overall agreement between the BD FACSCanto II and predicate was 98.8% and between the BD FACSCalibur and predicate was 99.1% (Table 8). Positive and negative agreements between the investigational and predicate methods indicated whether the samples were accepted or not by the investigational or predicate methods; notice that the percentage of positive and negative agreements is >96%, pointing out acceptable concordance. The number of discrepancies (rejected samples) was small, 5 for the BD FACSCanto II vs 6 for the predicate, and 6 for the BD FACSCalibur vs 2 for the predicate. Figure 4 displays the reported values around the clinical cut-off between 0 and 40 cells/μL for the investigational (y) and predicate (x) methods. Red lines intersect at the cut-off value (10 cells/μL), dividing the plot in four sections. Sections at the top right and bottom left show the positive and negative agreement between the investigational and predicate methods. The top left and bottom right sections show disagreement between the two methods.

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Evaluation of Inter-site Variability

Inter-site variability was evaluated by investigational method and by site. Results are reported as the absolute differences for Abs CD34 and %CD34. The number of specimens analyzed by site was: DRK = 385; DUK = 175; PCT = 135; and RPC = 223. The Abs CD34 difference values were between 6.2 and −3.2 for the BD FACSCanto II and 5.1 and −3.3 for the BD FACSCalibur. Similarly, the %CD34 difference was between 0.12 and −0.03 for the BD FACSCanto II and 0.2 and −0.04 for the BD FACSCalibur. Table 9 shows details of the absolute difference of Abs CD34 and %CD34 by investigational method and study site.

Figure 5 illustrates the difference of Abs CD34 and %CD34 by investigational method and site in a parallel fashion. Notice that the mean biases in both investigational methods follow a similar trend by site.

Process Control

For the duration of the study, the BD SCE low and high process controls were prepared, tested, and found to pass QC criteria before specimen enrollment. Additional analysis was carried out on four lots of the BD SCE process controls used at three sites during four consecutive months. Each lot was used for seven or more days per month and prepared by two site operators. DRK used different lot numbers, and thus was not included in this analysis.

Results show that the values for low and high process controls were within the specified manufacturers' ranges. The coefficient of variation of the low control was between 7.8% and 16.7% and for the high control was 4.5% to 10.7%. This is lower than the 20% CV for sample preparation variability (Table 10).

Inter-site Variability by Specimen Type

There was a wide range of results within and between different sites for most specimen types. Despite these variations, there was a general agreement between sites, most clearly demonstrated by data generated from tissue types that were less affected by local practices in mobilization regimen, harvest technique, and timing of collection, among others. In this regard, fresh NPB and fresh CB provided the most comparable tissue for analysis, and the data show tight clustering of results for these specimens, using either the BD FACSCanto II or BD FACSCalibur instrument platforms. The NPB and CB viable CD34 absolute differences for the BD FACSCanto II and BD FACSCalibur by clinical site are shown in Table 11.

Not surprisingly, data for either instrument or any specimen type were tighter for Abs CD34 than for %CD34 determination. The higher variability observed on results from thawed specimens can be attributed to differences in laboratory methodologies for freezing and thawing. Figure 6 illustrates the distribution of the specimen bias for viable $CD34^+$ and %CD34 (x axis) by investigational method and by clinical site.

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Stability

Stability of the specimen and staining was evaluated by measuring age of blood (AOB) and age of stain. AOB was recorded (in hours) from the time of a fresh specimen collection or frozen specimen thaw to the start of staining, by adding the reagents to the specimen sample in a BD Trucount™ tube and mixing. AOB was reported in hours. The age of stain is defined as the length of time in minutes between the end of lysing the sample and transferring it onto wet ice and the transfer of the tube to the analyzer for the start of acquisition. This time was reported in minutes.

The fresh specimen AOB was determined by the investigational methods and predicate, including 624 and 638 BD FACSCanto II and predicate samples, respectively. In addition, 638 and 600 BD FACSCalibur and predicate samples, respectively, were analyzed.

The mean times for BD FACSCanto II and predicate were 12.1 and 12.3 hours, respectively, with a standard deviation (SD) of 8.6 hours for the two methods. The mean times for the BD FACSCalibur and predicate were 12.1 and 12.5 hours, respectively, with a standard deviation of 8.6 hours for both methods, with a range between 10 minutes and 24 hours from draw. The frozen specimen AOB was measured by pairing the investigational and predicate methods. There were 313 and 303 frozen specimens acquired on the BD FACSCanto II and predicate respectively, and 313 and 316 frozen specimens on the BD FACSCalibur and predicate, respectively. The mean for all samples was 30 minutes, with a 23-minute SD. Overall, the AOB of all frozen samples ranged between 0 minutes and 1 hour from thaw.

Age of stain was measured in a similar fashion; with 951 BD FACSCanto II and 927 predicate samples. The mean age of stain for the BD FACSCanto II and predicate were 17.1 minutes and 15.9 minutes, with SDs of 10.2 and 11.7 minutes, respectively. There were 951 BD FACSCalibur and 915 predicate samples. The means were 17.1 minutes and 13.8 minutes, with SDs of 0.2 minutes and 10.1 minutes for the BD FACSCalibur and predicate, respectively. The range was 1 to 54.4 minutes.

Discussion

This multisite prospective study was designed to evaluate the performance of the novel BD SCE Kit, a single platform, BD Trucount based assay with a viability dye, applying the modified ISHAGE gating strategy for stem cell enumeration on two flow cytometry platforms at four clinical sites. The total number of specimens enrolled in the study exceeded the minimum number required to maintain a well balanced enrollment scheme at the four clinical sites. Furthermore, eight specimen types were tested in sufficient numbers to provide statistical power for analysis (Table 2), using common anticoagulants either alone or in combination (Table 3). The BD SCE Kit initially was marketed in Europe following completion of the EPE at the DRK. Their results showed equivalent performance between the BD SCE Kit and BCI Stem-Kit (14) within a single site.

The later incorporation of the three sites in the US addressed potential concerns and risks related to differences in expertise and methods in stem cell enumeration between the clinical sites. Despite the selection for inclusion in this study of institutions having experienced flow

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cytometry staff, we felt that given this was such a large study involving four laboratories from around the world, in order to provide assurance that we were limiting any source of variability to the test article itself, it was critical to ensure that the ISHAGE Method was being consistently performed within and between each institution. These concerns, in addition to the robustness of the test method, were mitigated with training of the site staff participating in the study, and therefore addressed operator-dependent variability as previously raised (9, 17). Our results confirmed that methods for sample handling and preparation were equivalent between sites (Table 4). In a recent study, the BD SCE Kit was compared to the BCI Stem-Kit and the BD Procount™ kit using fresh and thawed specimens (15). The results from the BD SCE Kit correlate well with the data from the other commercially available diagnostic tests. Results from the EPE (14) showed equivalency between both the BD SCE Kit and BCI Stem-Kit, irrespective of the flow cytometry instrument used. The results are in linear correlation across the entire concentration range, with correlation coefficients in excess of R^2 >0.9 for all specimen types. The data from EPE were included for analysis. However, the analysis methods applied in the present results were based on the CLSI guideline methods (18), using the first replicate for data analyses, and the second replicate for outlier determination, supporting a single tube testing assay.

Overall our data have shown equivalent performance between the BD SCE Kit and the predicate in three different ways. First, the absolute and relative differences of the BD SCE Kit were within 95% CI on viable Abs CD34, %CD34, and Abs CD45. And, in each of the respective bins on the two BD flow cytometry platforms (Table 7), all results fell within the predefined acceptance criteria. Second, results also showed equivalency between the BD SCE Kit and BCI Stem-Kit in the Deming linear regression, with correlation coefficients of R^2 ≥0.92 for Abs CD34, %CD34, and Abs CD45 (Figure 2). Third, our results also show the overall agreement was >98.8% with the predicate around the clinical cut-off criterion of 10 cells/μL, with >99% positive agreement and >96% negative agreement due to disagreement with ≤5 specimens, which otherwise the predicate method would have accepted (Figure 3). In summary, results of this multisite evaluation showed equivalent performance of the BD SCE Kit to the predicate, the BCI Stem-Kit.

Typically, a viable $CD34^+$ cell concentration equal to or higher than 2×10^6/kg has been considered an acceptable dose for successful hematopoietic engraftment and hematological reconstitution (19, 20). Therefore, although challenging, accurate enumeration of viable $CD34^+$ cells in products with low viable $CD34^+$ counts is critical, and results of the concordance analysis are particularly insightful around the clinical cut-off of viable $CD34^+$ of 10 cells/μL, ie, the dose thought to be a clinically relevant indicator of $CD34^+$ cell mobilization (20). To ensure acceptable measurement of viable $CD34^+$ cells in specimens with low numbers of viable $CD34^+$ cells, the algorithm of the BD SCE Kit has integrated three levels of quality control, requiring a minimum number of viable $CD45^+$ events (75,000), a minimum number of viable $CD34^+$ events (100), and a minimum number of beads (1,000). Should any of these three criteria not be met, the acquisition time is automatically extended to 15 minutes, increasing the probabilistic chances of obtaining an acceptable measurement of viable $CD34^+$ cells.

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Interestingly, our data, examined by site, show that the Abs CD34 biases by site and by method are located on the positive or negative section of the plot, and this location is consistent in both BD platforms. For example, DRK and RPC had a tendency for a slight negative bias, whereas DUK was closer to zero and PCT had a slight positive bias (Figure 5). However, the differences between sites fell within the acceptable 10%. The data analyzed by specimen type showed a linear correlation with R^2 >0.9, even though fresh and thawed specimens were pooled for analysis (Figure 3). Additional analyses by site on less manipulated specimens during the collection process, NPB and fresh CB, depicted the sites' biases as clusters, suggesting consistency of the testing methods (Figure 6). Interestingly, fresh LPH and fresh BM exhibited more variability, which might be intrinsic to the specimen and to methods for specimen collection. Lastly, thawed specimen (LPH, BM, and CB) biases fell within the predetermined specifications, although they exhibited more variability compared to fresh specimens.

Our results showed site equivalency for specimen handling and sample preparation using the BD SCE Kit and provide an insight into the robustness of the data obtained from all participating clinical sites. However, further intra-site comparison was confounded by the limitation of not having the same samples tested at all four clinical sites, which would have provided supporting evidence concerning the BD SCE Kit inter-site performance. In this regard, the analysis of the four lots of BD process controls at the three US sites provided encouraging results, since results show a CV within 20% for the low control with CD34 cell concentration around 10 cells/μL, these results do not replace the inter-site precision comparison.

Accurate enumeration of $CD34^+$ cells is a critical parameter for stem cell transplantation for clinical and therapeutic purposes. The single platform ISHAGE method with viability dye has been embodied in several guidelines (12, 21, 22) contributing to global standardization of this procedure, reducing interlaboratory variation to CVs of <10% in laboratories participating in the United Kingdom National External Quality Assessment Service for Leucocyte Immunophenotyping $CD34^+$ study (23). A similar strategy also has been used by the New York State Department of Health (24), although it recommends the use of Life Technologies TO-PRO® dyes for cell viability analysis. The BD SCE Kit gating strategy is based upon the single platform ISHAGE protocol (8,12), with the advantage of a single-tube assay. Our results show that, as long as the $CD34^+$ testing is done following the BD SCE Kit instructions, results are reproducible.

There is evidence showing that viability of $CD34^+$ cells from PBSC and BM has different profiles. The PBSC $CD34^+$ cell viability rapidly declines at room temperature. When cells are refrigerated (2°–8°C), PBSC $CD34^+$ viability is slowly reduced at 48 and 72 hours. The same conditions have a limited effect on CD34 viability of BM cells (25), while other factors that affect cell viability include shipping, storage conditions, and cell concentration (26).

Laboratories involved in minimally manipulated tissue banking receive most of the specimens collected (ie, CB) from diverse institutions, which sometimes could be international centers. The public Cord Blood Banks for the most part follow the international

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standards and the FDA guideline (27, 28) for processing and banking of specimens within the 48-hour window before freezing. The results from our study are limited to specimens no older than 24 hours that meet the criteria of the cleared BD SCE Kit product labeling. This might be a limitation for adoption of the BD SCE Kit by new customers, since it might require additional internal validation activities for those specimens older than 24 hours.

Selection of the appropriate product for transplantation is determined by multiple parameters, one of which is accurate measurement of viable $CD34^+$ cells. However, there are additional assays that are part of testing typically applied as a reasonable predictor of specimen potency and successful engraftment, such as total nucleated cells and the widely used colony forming unit assay, which have been proposed as endpoint(s) of engraftment potency (29, 30, 31). Other laboratories have included markers for T cells for allogeneic peripheral blood stem cell transplantation to reduce graft versus host disease (32) and markers to evaluate pre-apoptotic (24) and apoptotic $CD34^+$ cells (33).

Conclusions

The results for this multisite accuracy study have shown agreement between both investigational methods and the predicate method. It was also demonstrated that there was high concordance to the predicate method for Abs CD34, %CD34, and Abs CD45. The BD SCE Kit is cleared for the use of simultaneous enumeration of viable dual-positive $CD45^+$/$CD34^+$ hematopoietic stem cell populations. Results are presented in $CD34^+$ absolute counts (cells/μL) as well as the percentage of the total viable leucocyte count that is $CD34^+$ (%CD34). Normal and mobilized peripheral blood, fresh and thawed LPH products, fresh and thawed BM, and fresh and thawed CB specimens can be analyzed with this kit. The kit is intended for in vitro diagnostic (IVD) use on either a BD FACSCalibur flow cytometer using BD CellQuest (v3.3) or BD CellQuest Pro software (v4.02, 5.2.1, or v6.0) or a BD FACSCanto II flow cytometer using BD FACSCanto clinical software (v2.4) with the BD SCE Application module.

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Abbreviations

7-AAD	7-aminoactinomycin D
ACD-A	anticoagulant citrate dextrose – solution A
AOB	age of blood
BCI	Beckman Coulter, Inc
BM	bone marrow

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BSA	bovine serum albumin
CB	cord blood
CPD	citrate phosphate dextrose
CRF	case report form
DRK	Deutsch Rotes Kreuz (German Red Cross)
DUK	Duke University
EDTA	ethylenediaminetetraacetate
EPE	European Performance Evaluation
FDA	Food and Drug Administration
HSPC	Hematopoietic stem progenitor cell
IRB/EC	Institutional Review Board/Ethics Committee
ISCT	International Society for Cellular Therapy
ISHAGE	International Society of Hematotherapy and Graft Engineering
LPH	leucopheresis
MPB	mobilized peripheral blood
NPB	normal peripheral blood
PB	peripheral blood
PBS	phosphate buffered saline
PBSC	peripheral blood stem cell
PCT	Progenitor Cell Therapy
QC	quality control
RPC	Roswell Park Cancer Institute
SCE	stem cell enumeration

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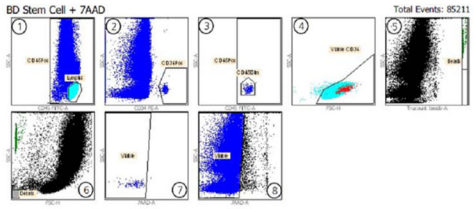
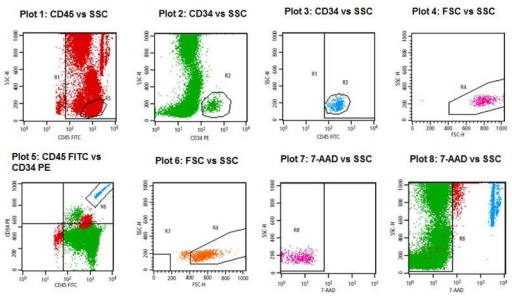
A



Plot no.	Gate(s)	Plot no.	Gate(s)
①	CD45Pos, Lymphs	⑤	Beads
②	CD34Pos	⑥	Debris
③	CD45Pos, CD45Dim	⑦	Viable
④	Viable CD34	⑧	Viable

B


Plot 1: CD45 vs SSC
Plot 2: CD34 vs SSC
Plot 3: CD34 vs SSC
Plot 4: FSC vs SSC
Plot 5: CD45 FITC vs CD34 PE
Plot 6: FSC vs SSC
Plot 7: 7-AAD vs SSC
Plot 8: 7-AAD vs SSC

Plot	Gates	Plot	Gates
1	Ungated plot includes all CD45dim to CD45bright events and excludes debris, platelets, and unlysed red blood cells (RBCs), which are all CD45–	5	Ungated multicolor plot is used to identify beads (R6)
2	Displays viable CD45 (G1) cells and is used to identify CD34+ cells. Adjust R2 to include all CD34+ events	6	Displays Viable Lymphs (R5 and R8) and is used to establish the minimum FSC and SSC ranges for R4
3	Displays G2 cells and is used to further define the viable CD34+CD45+ cell population	7	Contains the first gate to be adjusted and is used to identify viable cells (7-AAD–)
4	Displays G3 (R3 and G2) cells and is used to identify viable stem cells (CD34+)	8	Gated on the Total CD34 gate (R1 and R2 and R3) and is used during compensation optimization (FL3%–FL2)

Figure 1.

(A) The dot plots illustrate the ISHAGE modified gating strategy used in the BD SCE application module of BD FACSCanto clinical software. Plot 1: CD45 positive lymphs gate; Plot 2: CD34 positive; Plot 3: CD45 positive and CD45 dim gate; Plot 4: Viable CD34; Plot 5: Bead gate; Plot 6: Debris gate; Plot 7: Viable total CD34; Plot 8: Viable cells.

(B) SCE template for BD CellQuest software. Plot 1: $CD45^{dim}$ to $CD45^{bright}$ events; Plot 2: viable CD45 (G1) cells; Plot 3: G2 defining the viable $CD34^{+}CD45^{+}$ cells; Plot 4: G3 (R3 and G2) to identify viable stem cells ($CD34^{+}$); Plot 5: beads; Plot 6: viable lymphs (R5 and R8); Plot 7: Total CD34 gate; Plot 8: viable cells ($7\text{-}AAD^{-}$).

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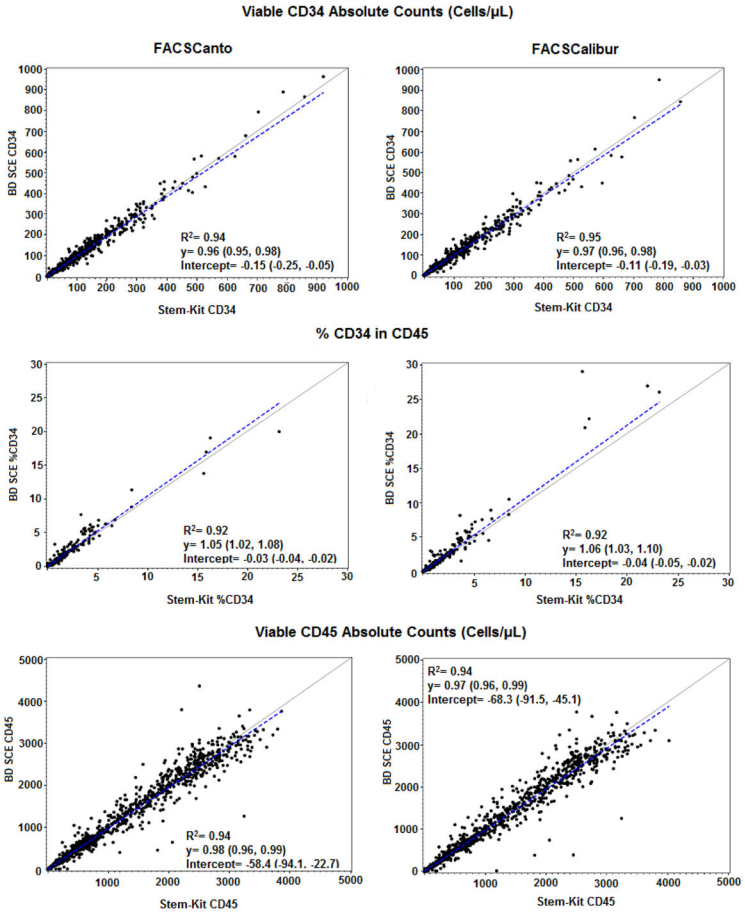

Figure 2.

Regression analysis with Deming fit of Abs CD34 (cells/ µL; top), %CD34 (middle), and Abs CD45 ((cells/ µL; bottom). The BD FACSCanto II method is on the left side, and the BD FACSCalibur method is on the right side. The investigational method is represented on the vertical (y) axis. Values on the horizontal (x) axis represent the predicate method. The solid line corresponds to the identity line, and the blue dotted line is the regression line. Data show a linear correlation with an R^2 >0.92 and intercept within 95% CI.

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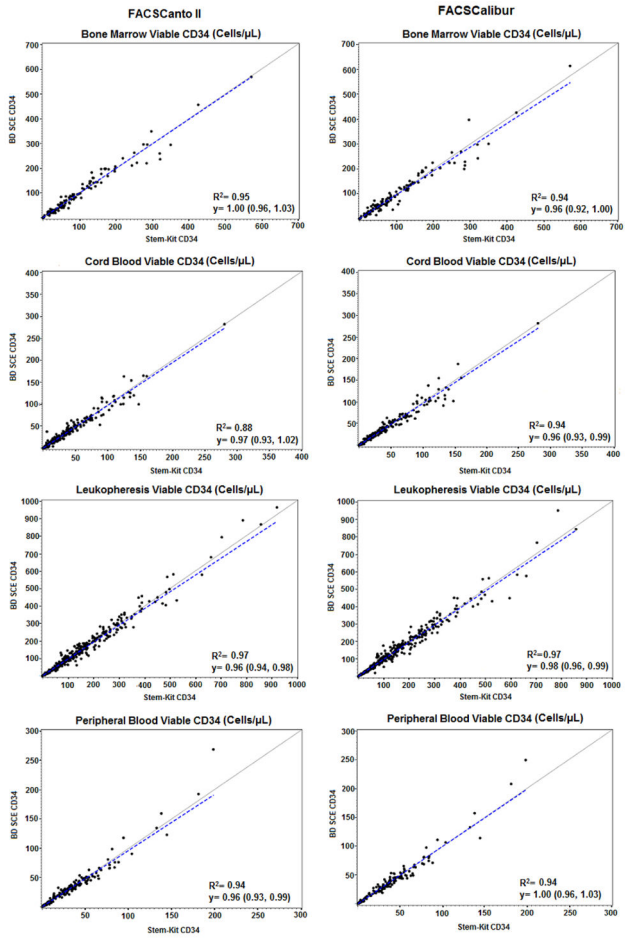

Figure 3.

Regression analysis with Deming fit for viable Abs CD34 (cells/ µL) by method (left, BD FACSCanto II; right, BD FACSCalibur) and specimen type. Fresh and frozen BM, CB, and LPH specimens were pooled for analysis. The predicate method is represented on the horizontal (x) axis, and the investigational method is on the vertical (y) axis. The solid line corresponds to the identity line, and the blue dotted line is the regression line. Data show a linear correlation with an R^2 >0.90 and intercept within 95% CI.

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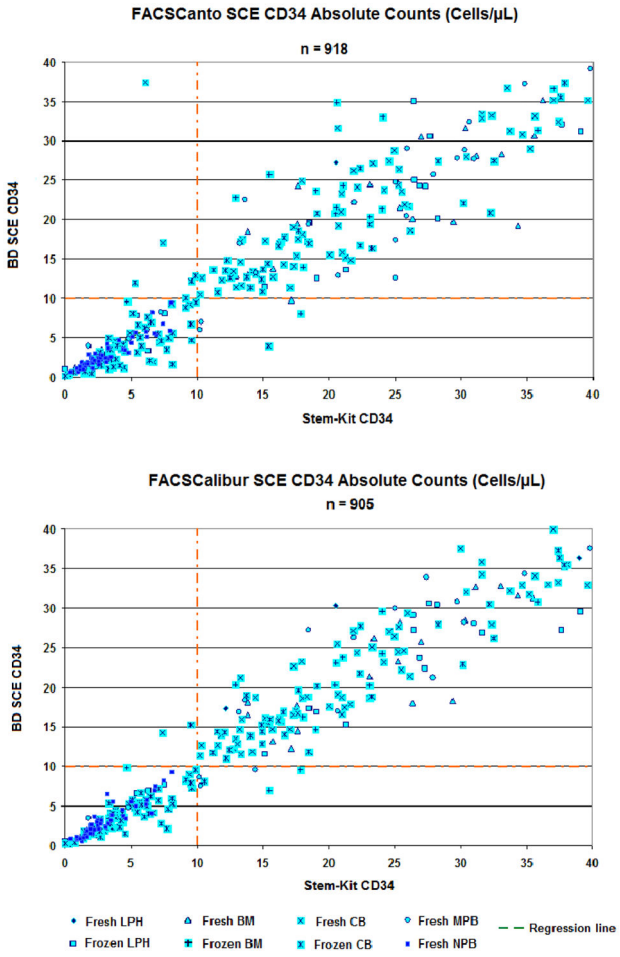

Figure 4.

Concordance analysis illustrates the reported values by investigational (y) and predicate (x) methods between 0 and 40 cells/µL. Red lines intersect at the cut-off value (10 cells/µL), dividing the plot in four sections. The top right section represents the positive agreement and the bottom left section shows the negative agreement between the two methods. The top left section is the investigational method disagreement, and bottom right section depicts predicate disagreement.

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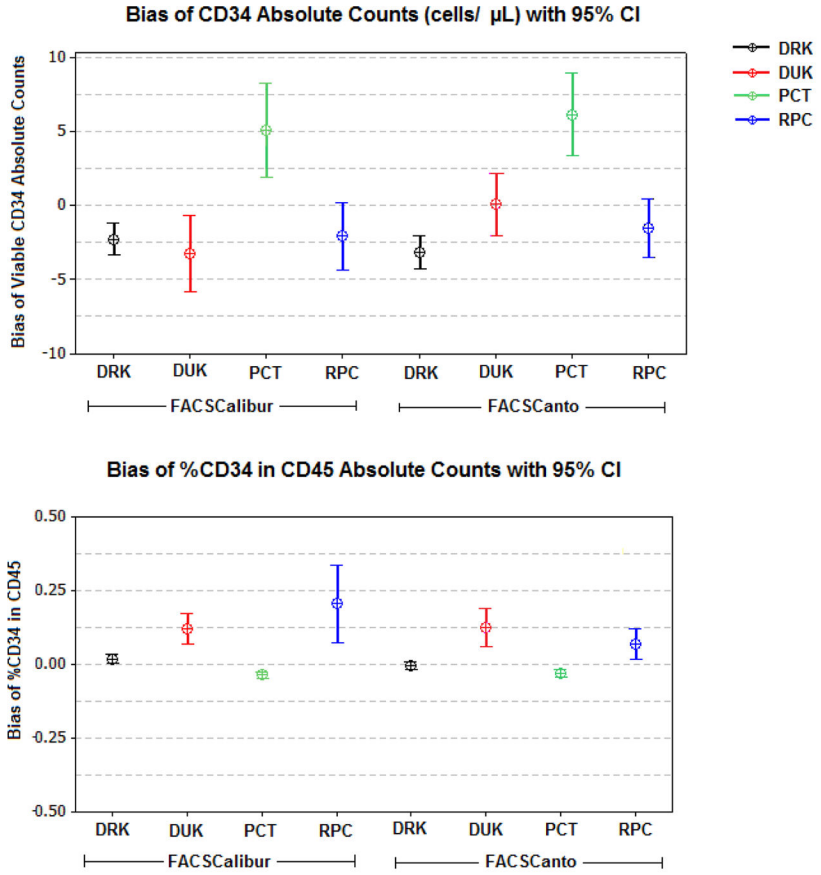

Figure 5.

Absolute differences of viable CD34 (cells/ µL) and %CD34 in CD45 with 95% CI by method and clinical site.

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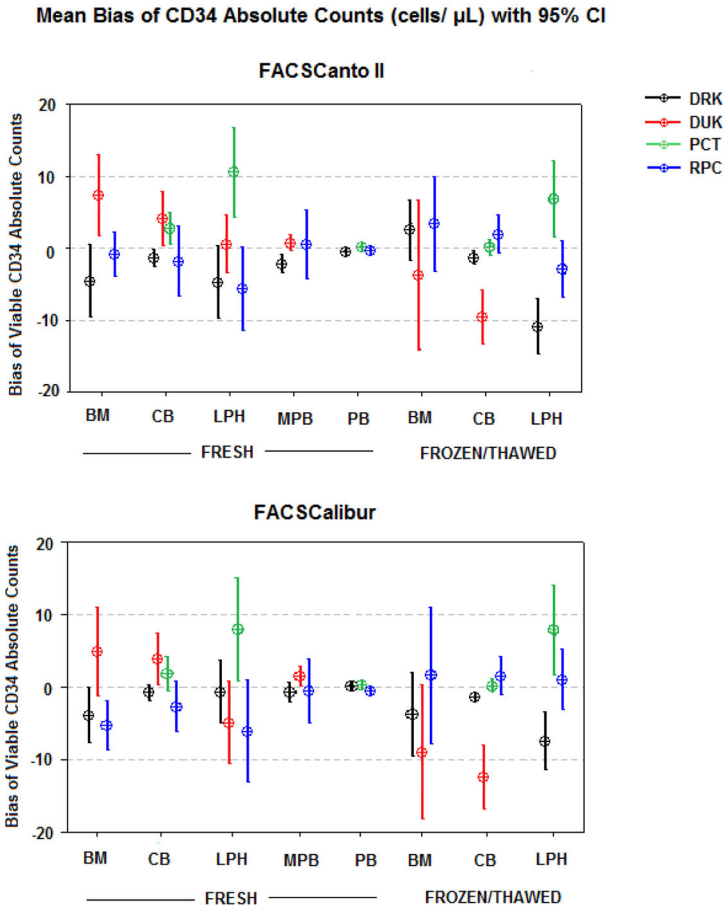

Figure 6.

Absolute differences of viable CD34 (cells/ µL) with 95% CI for the BD FACSCanto II and BD FACSCalibur shown by clinical site and specimen type. The BD FACSCanto II (top) and BD FACSCalibur (bottom) absolute difference for each clinical site is shown by different colors: DRK in black, DUK in red, PCT in green, and RPC in blue.

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Table 1

Summary of the Study Methods, Reagents, Analyzer Configuration, and Software Versions

Method	Reagent Kit	Cytometer	Software	Analysis
Predicate	BCI Stem-Kit	BD FACSCanto II, 5/3	BD FACSDiva™ v6.1.1 (EU) v6.1.3 (US)	BCI Template v1.5 (EU) v1.6 (US)
Investigational	BD SCE	BD FACSCanto II, 5/3	BD FACSCanto™ v2.4	Stem Cell Module v1.0
	BD SCE	BD FACSCalibur	BD CellQuest™ Pro v6	BD SCE Template v1.0 (EU) v1.0.1 (US)

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Table 2

Type of Specimens Tested by Investigational Method

Specimen Type	Total BD FACSCanto II	Total BD FACSCalibur
Fresh BM	75	73
Fresh CB	124	122
Fresh LPH	232	232
Fresh MPB	131	115
Fresh NPB	57	52
Frozen BM	73	78
Frozen CB	117	123
Frozen LPH	109	110
Total	918	905

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Table 3

Type of Anticoagulants Tested by Investigational Method

Anticoagulants	BD FACSCanto II	BD FACSCalibur
CPD	85	85
ACD-A	162	163
Heparin	180	188
Heparin + ACD-A	1	1
EDTA	472	450
EDTA + ACD-A	18	18
Total	**918**	**905**

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Table 4

Absolute number of viable CD34 (cells/μL) in Normal Peripheral Blood and Mobilized Peripheral Blood

Fresh NPB		**Investigational**		**Predicate**	
	N	**Mean**	**SD**	**Mean**	**SD**
BD FACSCanto II	57	2.8	1.9	3.1	2.0
BD FACSCalibur	52	3.0	1.9	3.0	1.9
Fresh MPB		Investigational		Predicate	
	N	Mean	SD	Mean	SD
BD FACSCanto II	131	40.1	35.0	40.9	31.8
BD FACSCalibur	115	45.2	32.6	45.2	32.6

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Table 5

Absolute number of viable CD34 (cells/μL) in Fresh and Frozen Bone Marrow, Cord Blood, and Leucopheresis Specimens

BM		**Investigational**		**Predicate**	
Fresh	**N**	**Mean**	**SD**	**Mean**	**SD**
BD FACSCanto II	75	80.2	73.6	80.1	73.4
BD FACSCalibur	73	83.2	72.6	83.2	72.6
Frozen					
BD FACSCanto II	73	89.9	108.1	88.8	108.7
BD FACSCalibur	78	89.0	108.1	89.0	108.1
CB		Investigational		Predicate	
Fresh	N	Mean	SD	Mean	SD
BD FACSCanto II	124	42.0	39.3	41.7	39.0
BD FACSCalibur	122	42.1	39.2	42.1	39.2
Frozen					
BD FACSCanto II	117	27.0	35.5	29.1	37.3
BD FACSCalibur	123	29.6	37.2	29.6	37.2
LPH		Investigational		Predicate	
Fresh	N	Mean	SD	Mean	SD
BD FACSCanto II	232	185.7	155.7	185.6	150.8
BD FACSCalibur	232	184.2	145.4	184.2	145.4
Frozen					
BD FACSCanto II	109	73.6	61.7	78.2	61.5
BD FACSCalibur	110	78.4	61.1	78.4	61.1

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Table 6

Relative Difference by Site and Method for Site Equivalency

	DRK	DUK	PCT	RPC	Max-Min
N	56	61	55	60	
BD FACSCanto II	−2.81	−0.5	4.31	−1.75	7.1
N	56	62	55	59	
BD FACSCalibur	−1.69	−2.69	5.2	−0.89	7.9

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Table 7

Viable Abs CD34 (cells/μL) Results by Bin and Method (All Specimen Types)

		Investigational		Predicate		Absolute Difference	
Low Bin							
Method	**N**	**Mean**	**SD**	**Mean**	**SD**	**Mean Bias**	**SD (95% CI)**
BD FACSCanto II	167	3.8	4.0	3.9	2.7	−0.1	1.4 (−0.5–0.3)
BS FACSCalibur	156	3.5	2.9	3.8	2.7	−0.2	1.4 (−0.4, −0.05)
Mid Bin							
BD FACSCanto II	496	43.3	26.3	44.0	24.5	−0.7	8.9 (−0.5, −0.3)
BS FACSCalibur	492	45.0	24.4	44.4	26.3	−0.2	1.4 (−0.4, −0.05)
High Bin							
BD FACSCanto II	255	217.1	135.0	218.5	128.8	−1.4	28.7 (−4.3, 1.6)
BS FACSCalibur	257	217.0	123.1	213.5	126.2	−3.6	31.5 (−6.8, −0.3)

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Table 8

Concordance Analysis around the Clinical Decision Cut-off (10 cells/μL)

Decision = 10 cells/μL		Accept	Reject	Total	Overall Agreement	Positive Agreement	Negative Agreement
BD FACSCanto II	Accept	757	6	763	98.80%	99.30%	96.20%
	Reject	5	150	155			
	Total	762	156	918			
BD FACSCalibur	Accept	753	2	755	99.10%	99.20%	98.60%
	Reject	6	144	150			
	Total	759	146	905			

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Table 9

Absolute Difference of Viable CD34 (cells/μL) and %CD34 by Investigational Method and Study Site

		Absolute Difference		
	Site	**Mean Bias**	**SD**	**95% CI**
	Viable			
FACSCanto	DRK	−3.2	13.2	(−4.3, −2.08)
	DUK	0.1	17.1	(−2.1, 2.2)
	PCT	6.2	19.7	(3.4, 9.0)
	RPC	−1.5	17.9	(−3.5, 0.5)
FACSCalibur	DRK	−2.3	12.5	(−3.4, −1.23)
	DUK	−3.3	20.5	(−5.8, −0.7)
	PCT	5.1	22.2	(1.9, 8.3)
	RPC	−2.1	21.1	(−4.4, 0.2)
	% CD34 in CD45			
FACSCanto	DRK	−0.01	0.2	(−0.02, 0.01)
	DUK	0.12	0.5	(0.06, 0.19)
	PCT	−0.03	0.1	(−0.05, −0.02)
	RPC	0.07	0.5	(0.01, 0.12)
FACSCalibur	DRK	0.02	0.2	(0.002, 0.03)
	DUK	0.1	0.4	(0.1, 0.2)
	PCT	−0.04	0.1	(−0.05, −0.03)
	RPC	0.2	1.2	(0.07, 0.3)

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Table 10

Comparison of the BD Low and High Process Controls at Three Clinical Sites

Lot Number	High				Low		
	Site	Mean	CV (%)	Specified Range	Mean	CV (%)	Specified Range
BC011H	DUK	32.9	8.5	24.2–41.4 $CD34^+$ cells/μL	8.6	12.2	5.3–12.1 $CD34^+$ cells/μL
	PCT	31.9	9.2		8.5	16.7	
	RPC	29.1	5.8		8.0	7.8	
BC021H	DUK	33.9	4.5	19.8–46.4 $CD34^+$ cells/μL	8.2	9.2	4.4–12.6 $CD34^+$ cells/μL
	PCT	33.9	8.9		8.7	13.4	
	RPC	29.5	6.4		7.3	9.4	
BC110H	DUK	33.2	7.6	23.6–43.6 $CD34^+$ cells/μL	11.6	11.0	8.0–14.4 $CD34^+$ cells/μL
	PCT	32.2	8.4		11.4	10.8	
	RPC	30.4	10.7		10.8	9.2	
BC120H	DUK	35.3	7.9	28.3–46.4 $CD34^+$ cells/μL	10.8	11.2	7.3–14.5 $CD34^+$ cells/μL
	PCT	35.2	6.0		10.7	10.1	
	RPC	31.9	6.9		9.5	12.7	

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Table 11

Absolute Difference of Viable CD34 (cells/μL) in Fresh Cord Blood and Normal Peripheral Blood

		BD FACSCanto II		BD FACSCalibur	
	Site	**N**	**Mean Bias (95% CI)**	**N**	**Mean Bias (95% CI)**
Fresh NPB	DRK	29	−0.5 (−0.7, −0.3)	27	0.1 (−0.1, 0.3)
	DUK	14	0.2 (−0.1, 0.4)	12	0.2 (−0.4, 0.8)
	RPC	14	−0.3 (−0.7, 0.1)	13	−0.6 (1.0, −0.2)
Fresh CB	DRK	60	−1.3 (−2.5, −0.1)	58	−0.8 (−1.9, 0.3)
	DUK	23	4.2 (0.4, 7.9)	23	3.8 (0.2, 7.4)
	PCT	20	2.9 (0.6, 5.1)	20	1.8 (−0.6, 4.2)
	RPC	21	−1.7 (−6.6, 3.2)	21	−2.7 (−6.2, 0.7)